Comparative study of RNA extractions for functional analysis in a molecular genetics laboratory

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Study objectives

Gene expression analysis using RNA isolated from whole blood is an innovative diagnostic procedure. Various pre-analytical barriers need to be overcome, such as transcript instability, and ex vivo gene induction is increasingly used in laboratories to study VUS (variants of unknown significance) diagnosed in the course of high-throughput sequencing. There is a variety of blood collection tubes available on the market.

However, the blood sampling devices that are currently available have certain limitations when it comes to RNA stabilisation and/or extraction procedures. SARSTEDT has developed a S-Monovette® tube that preserves RNA in blood lysed at the time of collection for at least 5 days at room temperature, at least 14 days at 8 °C and at least 36 months at -80 °C, while maintaining the integrity of the RNA. We compared the quality and quantity parameters of this new S-Monovette® tube with the commonly used PAXgene® Blood RNA tube (Becton Dickinson).

When a VUS (variant of unknown significance) is identified in DNA, it is possible to confirm the impact of the variant and determine the diagnosis (significance) by highlighting its effect on RNA using high-throughput sequencing.

This technique has a crucial pre-analytical impact on the results of functional analyses of samples.

Objective: to assess whether the new S-Monovette® RNA Exact tube can streamline and simplify clinical blood sampling and transit and extraction of RNA in the laboratory, while preserving the quality and quantity of extracted RNA.

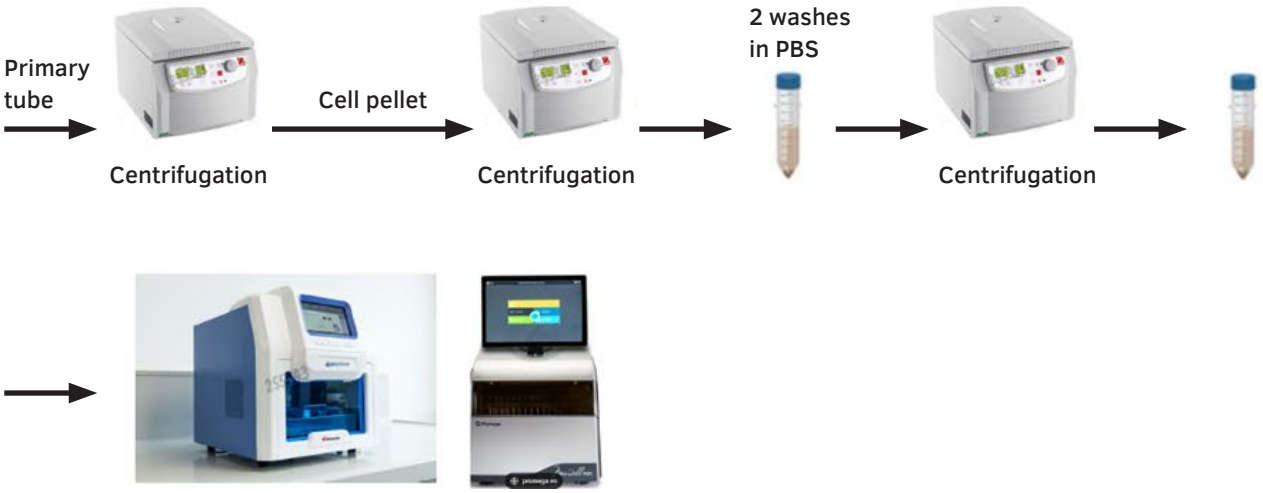
Pre-analytics

1. Protocol

A total of 16 patients were sampled with:

- 2 PAXgene® tubes (as usually requested in the clinical department)
- 1 tube S-Monovette® RNA Exact SARSTEDT

Extraction from PAXgene® BD tube:
PAXgene® red/transparent stopper: centrifugation with brake to gently "pellet" the WBCs (30 min) + 2 pellet washes in PBS (2x30 min) + pellet recovery in MR1 + TCEP, followed by Magnetapure extraction with RNA kit; **NucleoMag® RNA ref: 744350.1 MACHEREY-NAGEL or Maxwell**



Technical time: 3–4 h

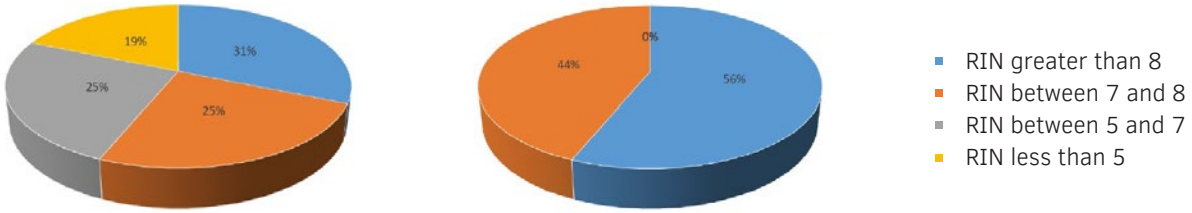
Extraction from S-Monovette® RNA Exact SARSTEDT tube:
SARSTEDT RNA Exact green cap: 850µlX3 whole blood +pk (15 min) + buffer, capture of NA nucleic acids on bead rack (large volume) (5 min), followed by extraction using Magnetapure automated system with special, dedicated RNA kit; NucleoMag® RNA blood ref: 744352.1 MACHEREY-NAGEL.

2.5 ml whole blood +pk + buffer (15 min), capture of NA nucleic acids on bead rack (large volume)

Technical time: 1.5 h

2. Results

RIN scores (N=16): The quality of RNA extracted from S-Monovette® RNA Exact is on average superior to the quality of RNA extracted from the PAXgene® tube.



Distribution of RIN scores / Paxgene tubes

Distribution of RIN scores / S-Monovette® RNA Exact tubes

Fig 1: RNA integrity number

Genomic DNA (N=16): Genomic DNA contamination is lower in RNA with the S-Monovette® RNA Exact tube (Fig 2).

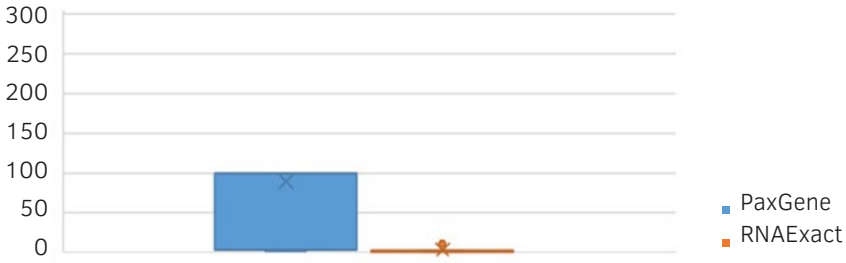


Fig 2: Measuring parasitic genomic DNA with QUBIT DNA

NGS result comparison
Rapid prenatal EXOME (Fig 3)

RNA Exact:
- Easy to read NGS data
- Parasite gDNA contamination is very low
- Patient diagnosis could be made

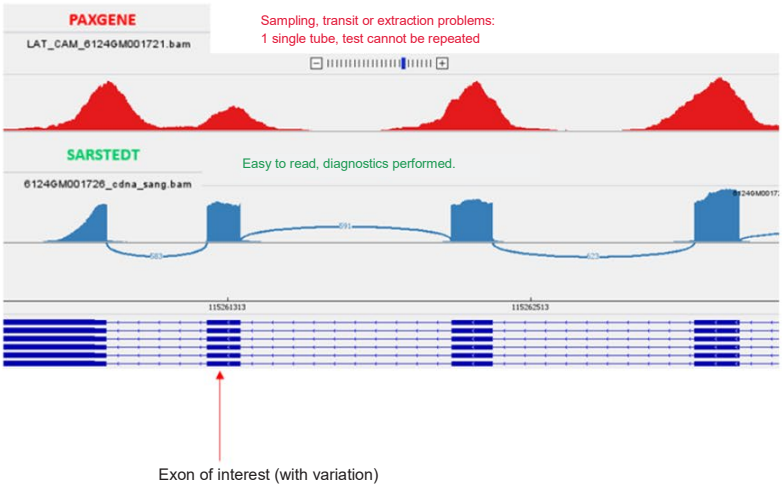
PAXgene®: failure:
Very high gDNA contamination made it impossible to read the transcripts. The exome searched for was illegible (problem suspected during pre-analytical phase or during RNA extraction).

NGS MCN: neurocortical disease

RNA Exact:
- Pathogenic pseudoexon
- Also very low incidence of **non patho pseudoexon** found in parents and other patients tested.

PAXgene®:
Pathogenic pseudoexon detected, **no other events**

NGS Metabolism (Fig 4)
Same reading with PAXgene® and RNA Exact, with identical interpretation and application.



Sashimi plot visualisation shows the same: genomic DNA contamination of cDNA with the Paxgene tube and exon/exon junction analysis impossible, whereas the SARSTEDT analysis shows very clear exon/exon junctions in ample quantity. For this project, we were able to conclude that the patient's exon/exon junction profile is identical to that of the control(s) on identical tissue. In addition, the good depth allowed us to conclude in favour of the absence of NMD (nonsense-mediated decay) since the variation identified by DNA genome sequencing has a cDNA VAF (variant allele frequency) of 0.5.

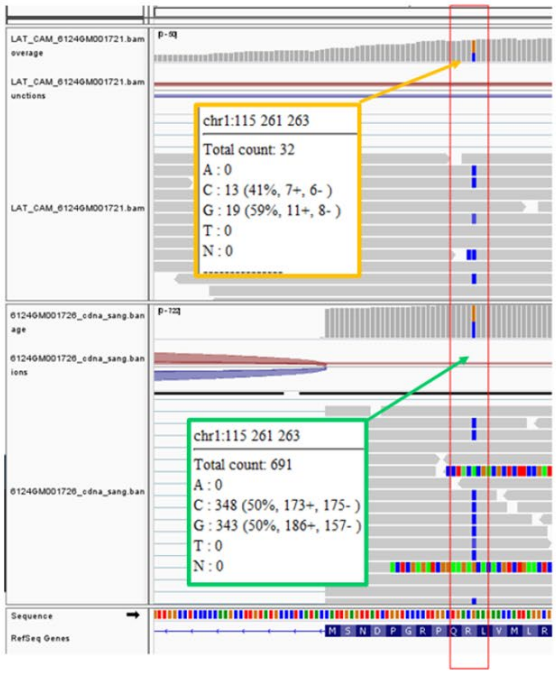


Fig 3: Prenatal exome

Patient with propionic acidemia
Study of gDNA 1 variant c.1057G>T (p.gly353*) in heterozygous state in exon 10 of PCCB gene No 2nd variant
Study of PCCB mRNA: Paxgene Blood RNA and S-Monovette® RNA Exact tubes

Same reading with PAXGENE and SARSTEDT, with identical interpretation and application:

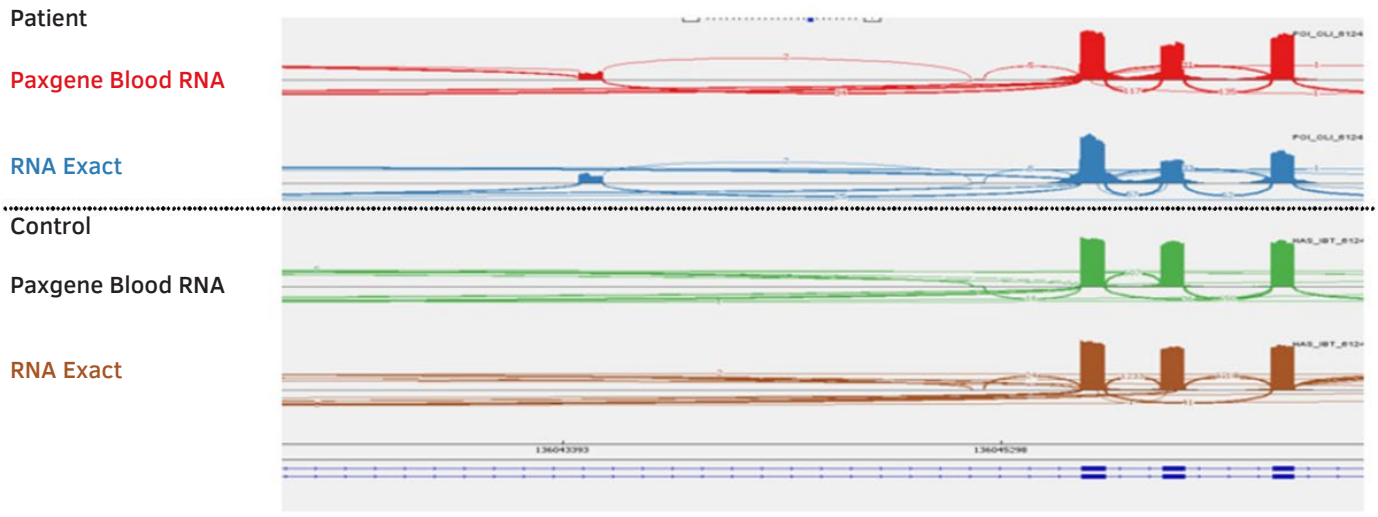


Fig 4: NGS Metabolism

3. Conclusion

- The results show that the quality and quantity of RNA extracted from the new S-Monovette® tube are better than or at least equivalent to that extracted from the PAXgene® tube, with consistent biological analysis results (exome and NGS)
- The extraction process is significantly shorter and simpler
- Less blood is drawn and inherent biological waste is reduced
- Simplified sample logistics: longer expiration dates and sample stability
- Suitable for all types of vein (vacuum and suction)

These results mean that it is now possible to routinely analyse patients‘ RNA using the new S-Monovette® tube, which is becoming increasingly important in molecular genetics laboratories as high-throughput sequencing and genome-wide analysis become more widespread.

The Molecular Genetics Laboratory at the Necker Hospital has validated the use of S-Monovette® RNA Exact SARSTEDT tubes for functional analysis.

If you have any questions, we'll be happy to help!

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